Effects of Remote Ischemic Conditioning on Hand Engagement in individuals with Spinal cord Injury (RICHES): A Preliminary Crossover Study

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Study Protocol

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Abstract

Background

Most spinal cord injuries (SCI) are not full transections, indicating that residual nerve circuits are retained after injury. SCI rehabilitation interventions, including physical training and neural stimulation, have been shown to beneficially reorganize motor pathways in the brain, corticospinal tract (CST), and at the spinal level. However, both physical training and neural stimulation require a large number of repetitions, and the retention of the intervention effects may be transient. Therefore, the need remains for an effective approach to synergistically improve the amount and duration of neuroplasticity in combination with other interventions. Remote ischemic conditioning (RIC) demonstrates several potential advantages as a candidate for such an approach. In this proposed study, we will investigate RIC coupled with physical training to promote neuroplasticity in hand muscles after cervical SCI This will be the first study to introduce RIC in the SCI population, so we will extensively focus on safety, tolerability, and hemodynamic responses during RIC.

Methods

This is a prospective randomized-order crossover trial to be performed over 24 months in 16 participants including 8 healthy controls and 8 participants with chronic cervical SCI. Patients will participate in one screening session (SCI subjects only) and two experimental sessions consisting of either active or sham RIC preceding a bout of pinch movement exercise. Serial evaluations will be carried out at baseline, after RIC, immediately after pinch exercise, and follow up 15-minutes later. The primary outcome is the change in corticospinal excitability (primarily measured by the motor evoked potential of abductor pollicis brevis muscle). Secondary outcomes will include maximal volitional pinch force, and inflammatory biomarkers such as components of the TLR signaling pathway. To ensure safety, we will monitor tolerability and hemodynamic responses during RIC.

Discussion

This study is the first to test RIC in people with cervical spinal cord injury and to investigate whether RIC alters corticospinal excitability. By sharing the details of our research protocol, we hope other interested researchers will seek to investigate similar approaches – depending on overlap with the current study and mutual sharing of participant-level data, this could increase the sample size, power, and generalizability of the analysis and results.

Background And Rationale

Between 250-350,000 individuals live with chronic spinal cord injury (SCI) in the United States. Among this population, ~ 60% have injuries at the cervical level[1]. Impairments of arm and hand function in individuals with cervical SCI (cSCI) greatly reduce quality of life and adversely impact the level of independence[2]. Previous research on the needs of individuals with cSCI has shown that improvement of

hand function is ranked more important than walking[3]. Most spinal cord injuries are not full transections, indicating that functional nerve circuits exist after injury[4]. Rehabilitation interventions such as physical training and neural stimulation have been shown to reorganize motor pathways in the brain, corticospinal tract, and at the spinal level to promote functional gains[5,6]. Thus, enhancing residual neural circuit excitability after SCI could improve hand function by increasing neuroplasticity[7–9].

However, both physical training and neural stimulation require a large number of repetitions, and even so, the retention of intervention effects may be transient. To lengthen retention of functional gains, investigators must find new ways to enhance the magnitude and duration of the neuroplastic effects. Acute intermittent hypoxia (AIH) is one such approach. Preliminary research has shown that AIH coupled with task-oriented physical rehabilitation enhances the learning effects of task-specific motor training after SCI, presumably through activation of raphe serotonergic neurons. This leads to intermittent spinal serotonin release, which further induces synthesis of brain-derived neurotrophic factor (BDNF) and activation of the high-affinity receptor tyrosine kinase, potentially leading to neuroplasticity[10]. However, AIH requires a hypoxicator system to provide prolonged systemic low oxygen exposure. Furthermore, AIH is costly, demands highly trained staff, and requires sophisticated technological devices. Therefore, the need remains for a simpler approach to synergistically improve neuroplasticity in combination with physical rehabilitation.

Ischemic conditioning occurs when a specific organ or tissue is exposed to one or more transient episodes of sublethal ischemia. This leads to several reactions that protect the organ system or tissue from subsequent ischemia[11–13]. Studies have demonstrated that these endogenous protective effects are not limited to the ischemic organ/tissue alone – they are transferrable to other organ systems or tissues, including the spinal cord[14–16]. This phenomenon is called remote ischemic conditioning (RIC)[17,18], and for example, cardioprotective effects have been reported by transiently restricting blood flow to one limb using a tourniquet. The simplicity of application and generalizability of effect makes RIC a more attractive, less expensive, and more practical approach to AIH.

The mechanisms of cardioprotection induced by RIC are not entirely clear, but evidence suggests that RIC has extensive effects via humoral and neural factors[18]. Among the potential mechanisms supporting the cardioprotective benefits of RIC, two might promote neuroplasticity: induction of trophic and anti-inflammatory factors. Hypoxia-inducible factor 1a (HIF-1a) may have neuroprotective effects via triggering the expression of genes related to oxygen transport, glycolytic metabolism, and apoptosis[19]. Albrecht and colleagues found that upper limb RIC induced H1F-1a accumulation and activation in right atrial tissue of patients undergoing cardiopulmonary bypass[20]. Vascular endothelial growth factor (VEGF) is another potentially neuroprotective factor shown to be induced by RIC [Ueno et al 2016 PMID 27905554]. Whether the activation of H1F-1a and VEGF induced by RIC extends to corticospinal areas and regulates neural excitability is unknown.

On the other hand, RIC may reduce systemic inflammation[21,22], which has been shown to attenuate expression of brain-derived neurotrophic factor (BDNF) in the brain[23–26]. One study demonstrated broadly elevated inflammatory gene expression in persons with chronic SCI, which was particularly evident in persons with higher level injuries[27]. Specifically, elevated expression of members of the Toll-like receptor 2/4 (TLR) signaling pathway, metalloproteinases (ADAM10), caspases (CASP1, 3, 8) and chemokine gene families were identified in individuals with SCI compared to able-bodied individuals. Studies in able-bodied adults showed that RIC reduced levels of inflammatory mediators in the blood 15 minutes and 24 hours later[28]. Of interest, members of the same genes and gene families elevated in persons with chronic SCI[27] were reduced by RIC in able bodied individuals: TLR signaling pathway, Tumor Necrosis Factor (TNF) receptor pathway, Mitogen-Activated Protein (MAP) kinases, apoptosis pathway (CASP8), chemokines, T cell signaling molecules, metalloproteinases and leukocyte adhesion moleculesref[27,28]

Cherry-Allen and colleagues recently published the first study testing the synergistic effects of RIC on motor task learning[29]. Able-bodied adults (n=18) were randomly assigned into active or sham RIC groups to undergo seven consecutive weekday sessions of RIC followed by stability platform balance training or training of a hippocampal-dependent cognitive task. Active or sham RIC (5 cycles of 5-min inflation and 5-min deflation) was conducted before training each day. The authors noted significantly improved performance on the stability platform task in the active RIC group compared to the sham group immediately after the completion of seven-day training programs; these effects were retained at the 2- and 4-week follow-up visits. These authors did not report significant changes in serum BDNF, cognitive learning, or generalized muscle activation measured by finger flexor EMG activity and grip force in the RIC compared to the sham group. The mechanism of improved balance was unclear, since they did not investigate changes of corticospinal excitability. In addition to monitoring safety and acute effects on grip force in this study we will focus mechanistically on the acute effects of RIC on corticospinal excitability and systemic inflammatory mediators.

We propose a proof of concept study to investigate the acute synergistic effects of active versus sham RIC with motor task training (isometric hand exercise) in persons with chronic cSCI. The primary outcome measure will be change in corticospinal excitability. Secondary outcomes will include pinch force and inflammatory biomarkers specifically on TLR signaling pathway. To ensure safety, we will monitor beat-to-beat changes in heart rate (HR), blood pressure (BP) and oxygen saturation (SaO_2) during RIC, since damaged autonomic nervous system (ANS) in individuals with SCI might dysregulate hemodynamic responses during the ischemic conditioning.

As far as we know, this will be the first study in the SCI population (1) to investigate the synergistic effects of RIC with physical training on corticospinal excitability, (2) to measure changes in inflammatory mediators after RIC, and (3) to observe in real time the responses of HR, BP and SaO2 during RIC.

Methods/design

Study design

This is an exploratory prospective randomized-order crossover trial to be performed over 24 months in 16 participants including 8 healthy controls and 8 participants with cSCI. In two separate sessions, active or sham RIC will be performed prior to isometric hand exercise. Participants with cSCI will undergo a screening session prior to the first experimental session to determine eligibility. Participants with chronic cSCI and able-bodied controls will be randomly assigned the order of the two experimental sessions: active or sham RIC. The isometric hand exercise will be performed in both sessions. Clinical and physiological measurements will be made during each session before and after sham/active RIC, and after isometric hand exercise. Blood samples will be drawn before and after sham/active RIC. The washout period between the two experimental sessions will be at least 14 days to prevent any carry-over effects.

Sample size

This project is the first to test RIC on people with SCI and investigate whether RIC alters nerve excitability in the pathway from the brain to the spinal cord to facilitate motor learning. For the primary efficacy outcome measure of corticospinal excitability, a sample size of 27 in each population would be needed to detect a generic medium effect size of 0.5 at a significance level of 0.05 with 80% power. Because this is a pilot study emphasizing safety and feasibility, we will enroll 16 participants (8 SCI and 8 able-bodied) to more specifically determine the effect size for subsequent efficacy trials.

Inclusion Criteria

Participants with SCI will be included if they fulfill the following inclusion criteria: (1) Age between 18 and 65 years; (2) Chronic (more than 12 months since injury) SCI between neurological levels C2-C8; (3) Score of 3 or more (out of 5) on manual muscle testing of finger extension, finger flexion, or finger abduction in left or right hand; (4) Detectable motor evoked potentials in left or right APB via transcranial magnetic stimulation (TMS); (5) Able to perform thumb-middle finger opposition pinch task with detectable pinch force. Additionally, able-bodied participants without any known central or peripheral neurological disease or injury will be recruited.

Exclusion Criteria

Participants will be excluded if they have any of the following: (1) Multiple spinal cord lesions; (2) History of seizures; (3) Use of medications that significantly lower seizure threshold, such as amphetamines and bupropion; (4) History of implanted brain/spine/nerve stimulators, aneurysm clips, or cardiac pacemaker/defibrillator; (5) Any other contraindication to undergoing magnetic resonance imaging (except for claustrophobia); (6) Significant coronary artery or cardiac conduction disease; (7) Open skin lesions over the arms; (8) Pregnancy; (9) Unsuitable for study participation as determined by study physician.

Screening Session

The purpose of this screening session is to ensure that participants with SCI meet all inclusion/exclusion criteria including detectable electrophysiological signals induced by TMS. Subjects will first undergo neurological examination according to the International Standards for the Neurological Classification of Spinal Cord Injury (ISNCSCI). The ISNCSCI neurological level of injury must be between C2-C8, with muscle strength of at least 3/5 in finger extension, finger flexion, or finger abduction in either hand. In order to qualify for the study, all participants must demonstrate the capability of performing a pinch grip between the tips of the thumb and third finger with detectable pinch force on at least one hand. Maximal volitional contraction (MVC) during thumb-third finger pinch will be recorded using a load cell dynamometer. Supramaximal electrical stimulation will be delivered at median and ulnar nerves at wrist level to induce maximal peak-to-peak amplitude of M wave (Mmax) and F wave. Recorded peripheral nerve parameters include the latency and the peak-to-peak amplitude of the M/F waves. After determining each participant's optimal scalp location for hand motor cortex stimulation using TMS (Magventure X100 with D-B80 coil) with neuronavigation (Brainsight). TMS will be performed to determine resting motor threshold (RMT), which will be defined as the lowest intensity at which at least 5 out of 10 stimuli result in a response of at least 50 uV from the targeted APB. If RMT cannot be detected at the APB muscle at stimulus intensity below 90% of maximal stimulator output, then active motor threshold (AMT) will be determined while the participant performs a pinch maneuver at 20% maximal effort. Participants will be ineligible for study participation if neither RMT nor AMT can be detected at the APB muscle at stimulus intensity below 90% of maximal stimulator output. If MEPs are detected, then an intensity-response curve will be recorded at 10-20% intervals between 100% and 200% of threshold in pseudorandom order. Peak-to-peak amplitude of 10 responses will be averaged per intensity. To account for possible changes in electrode placement and skin conductance over different testing sessions, MEPs will be normalized during each session to peripherally evoked Mmax[30].

Study session

Figure 1 depicts the experimental protocol. Each session will consist of a pre-test measurement (baseline), active or sham RIC, post-RIC measurement, an isometric hand exercise and post-exercise measurements. Blood samples (3cc) will be collected before the active/sham RIC cycle and 15 minutes after completion of active/sham RIC to measure changes in inflammatory mediators.

The RIC protocol involves 5 cycles of 5-min inflation and 5-min deflation on the non-target arm. The original RIC protocol used 200 mmHg inflation[17,31–35]; however, most individuals with cSCI are hypotensive, and may not need this high of a pressure for effective RIC. Cuff inflation to 20 mmHg above systolic blood pressure has been shown to have the same ischemic effects as 200 mmHg inflation pressure[36]. Therefore, in order to increase tolerability:

active RIC will be performed at a cuff inflation pressure of 20 mmHg above each participant's resting systolic blood pressure and

sham RIC will be performed at 10 mmHg below each participant's diastolic blood pressure.

During active and sham RIC, beat-to-beat HR, BP and SaO_2 will be monitored in real-time on the contralateral limb and digital signals will be stored on a computer hard-drive for subsequent analysis. A 3-lead ECG (UFI: model RESP 1, Morro Bay CA) will be used to monitor beat-to-beat HR during testing. Electrodes will be placed at the right and left clavicle and in the V-5 position; data will be recorded from V-5. Beat-to-beat BP will be continuously monitored from the target middle or ring finger using photoplethysmography (FMS: Finometer Pro; Amsterdam, Netherlands). Continuous SaO_2 will be monitored with a finger pulse oximeter, placed on the contralateral hand, which will be recorded at 1-minute intervals during administration of the active and sham RIC conditions. At the 4^{th} minute of each inflation period, participants will be asked to report any pain (on a scale from 0 to 10), discomfort, or other symptoms. Participants will be asked again at the 1^{st} minute of the following deflation period to check if the pain, discomfort, or other symptoms persist.

Reports suggest that performing volitional movements at varying intensities stimulates corticospinal circuits[37,38], as such, for the isometric hand exercise, participants will be instructed to pinch a dynamometer with tips of the thumb and third finger at different intensities and durations. Several studies have demonstrated immediate and transient increased MEPs as a result of post-exercise facilitation in able-bodied participants after a short period of repetitive contraction exercise in thenar[39], wrist[40], forearm[41], and leg muscles[42,43]. The various combinations of intensity and duration of pinch movements in our study, as well as the use of an intrinsic hand muscle, theoretically involves more cortical attention, which should magnify the likelihood of modulating supraspinal neuroplasticity[37,38,44]. Pinch force intensities will be randomized between 10%, 25%, and 50% of MVC, and the duration of contraction at each intensity will vary between durations of 2, 4, and 6 seconds, which results in nine different combinations delivered in pseudo random order. Participants will perform 2 sets of the 9 combinations of isometric hand exercise (18 pinches in total). The interval between each pinch task will be 2 seconds, with 30 second intervals between each set.

Electrophysiological variables and the maximal voluntary pinch force will be measured at pre-test measurement (baseline), post-active/sham RIC (post-RIC), and post-exercise (immediately after the isometric hand exercise (post-RICx-0) and 15 minutes later (post-RICx-15)).

Electrophysiological Outcomes

The primary muscles assessed in this study are APB and the first dorsal interosseous (FDI) muscle on the target arm.

Peak to Peak Amplitude of Motor Evoked Potentials at 120% intensity of Motor Threshold (MEP_{120}): A winged TMS coil is positioned over the optimal scalp location corresponding to the motor cortex area innervating the APB muscle of the target hand. The single-pulse TMS protocol to measure MEP_{120} amplitude comprises 10 unconditioned stimuli elicited at a stimulus intensity of 120% of the resting (or active) motor threshold as identified in the screening session. The MEP_{120} is a simple variable to measure the change of corticospinal excitability that has been used in multiple paired pulse and plasticity

experiments[45–47]. The 10-second inter-pulse interval (IPI) has been shown to have good reliability[48]. To account for possible changes in electrode placement and skin conductance over different testing sessions, MEPs are normalized during each session to peripherally evoked Mmax as previously described[30].

Recruitment curve: Also called input-output or stimulus-response curve, this indicates the increase in MEP amplitude with increasing TMS intensity. The recruitment curve is assumed to be associated with the strength and excitability of corticospinal projections[49]. In our protocol, the stimulus-response curve will be recorded at 10-20% intervals between 90% and 200% of threshold in pseudorandom order. Recruitment curves will be modeled using the Boltzmann Equation and parameters of the curve (Slope, Inflection Point) will be extracted for further comparisons[50].

Cortical inhibition and facilitation: Paired-pulse TMS will be used to measure short interval cortical inhibition (SICI), long interval cortical inhibition (LICI) and intra-cortical facilitation (ICF)[51]. Paired-pulse TMS includes a conditioning (CS) and test stimulus (TS) separated by a specified interstimulus intervals (ISI). The configuration for measuring SICI, LICI and ICF in this study are as follows[52–55]:

SICI: CS = 90% RMT, TS = 120% RMT, ISI = 3 ms

LICI: CS = 120% RMT, TS = 120% RMT, ISI = 100 ms

ICF: CS = 90% RMT, TS = 120% RMT, ISI = 12 ms

Ten paired-pulses will be obtained for each condition with an 8 second interval between each paired-pulse.

Peripheral Nerve Profile: Supramaximal electrical stimulation will be delivered to the median and ulnar nerves at wrist level. The peripheral nerve profile includes the latency and the peak-to-peak amplitude of the M/F waves. The peak-to-peak amplitude of the M waves (Mmax) will be used to normalize the MEP_{120} at each time point to ensure that changes of corticospinal excitability are not due to variation in recording electrode placement[51].

Pinch Force: Maximal voluntary thumb-3rd finger pinch force will be measured by a load cell dynamometer (100lb S-Beam load cell, ANYLOAD, New Jersey, USA) with customized 3D printed holder designed for pinch force measurement. Participants will perform three attempts of maximal pinch force and the best one will be recorded.

Data Analysis: MEP_{120} and intra-cortical facilitation/inhibition (SICI, LICI and ICF) at the target APB and FDI muscles post-RIC, and at 0 and 15 minutes post-isometric pinch exercise will be expressed as percentage change compared to baseline. The primary outcome will be **MEP_{120}** of APB muscle at the post-RICx-0 timepoint. **The MEP_{120}** will first be normalized to peak-to-peak amplitude of M waves (Mmax).

Statistical Analysis: Electrophysiological outcomes and pinch force will be compared after RIC and after the isometric hand exercise relative to baseline values. Descriptive analysis will be computed first for all outcome variables to test the distribution of the data and correlation tests will be performed to check the independence among outcome variables. A three way 2 (Group: cSCI, able-bodied) by 2 (Condition: RIC, sham) by 3 (Time: post-RIC, post-RICx-0, post-RICx-15) mixed-ANOVA will be used to analyze MEP120, intra-cortical facilitation/inhibition and pinch force. If the data is not normally distributed and the assumptions of the ANOVA are not met, Wilcoxon signed rank tests will be used instead. Post hoc pairwise comparisons will be performed using the Bonferroni adjustment to test significant pairwise comparisons following significant main or interaction effects among the three independent factors (Group; Condition; Time).

Gene expression of inflammatory biomarkers

RNA will be isolated from whole blood collected in PAXgene tubes (PreAnalytix, BD), using standard methods and the manufacturer's protocol (Qiagen QIAcube, Venlo, The Netherlands). RNA quality and quantity will be determined using the Bioanalyzer (Agilent). RNA will be converted to cDNA using the RT^2 First Strand Kit and RT^2 SYBR Green Mastermix. We will use the PCR Array for Human Toll-Like Receptor Signaling Pathway (Qiagen, USA) on the Roche Lightcycler 480 (384-well block).

Data Analysis:

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Statistical Analysis: Relative gene expression will be determined using the delta Ct method. Housekeeping genes included in the array are ACTB, B2M, GAPDH, HPRT1 and RPLP0; the one most empirically stable will be used as the reference control gene for normalization. A three-way 2 by 2 by 2 mixed model ANOVA will be performed for 2 within group factors (Time: baseline vs. post- RIC) (Condition: active vs. sham) and one between group factor (Group: able-bodied vs. cSCI) to compare changes in gene expression. Statistically significant differences ($P<0.05$) in gene expression will be corrected for multiple comparisons using the method of Benjamini and Hochberg, with a false discovery rate (FDR)=0.05.

Hemodynamic stability during RIC

During the 50-minute active and sham RIC (5 cycles of 5 min inflation plus 5 min deflation), beat-to-beat HR, BP and SaO_2 will be collected. The peak HR, BP and the minimal SaO_2 in baseline, inflation phase, deflation phase and post-RIC will be recorded for statistical analysis.

Data Analysis: During the 50-minute active and sham RIC (5 cycles of 5 min inflation plus 5 min deflation), beat-to-beat HR, BP and SaO_2 will be collected. The peak HR, BP and the minimal SaO_2 in baseline, inflation phase, deflation phase and post-RIC will be recorded for statistical analysis.

Statistical Analysis: A 3-factor mixed model ANOVA will be used to determine differences in HR, BP and SaO2 – 2 within group factor (Group; Condition) and one between group factor (Time). The time factor will include: 1) baseline, 2) average of the 5-inflation periods, 3) average of the 5-deflation periods and 4) post-RIC. Significant main or interaction effects will be further explored using Tukey post-hoc analyses. If the data is not normally distributed and the assumptions of the ANOVA are not met, Wilcoxon signed rank tests will be used instead. Post hoc pairwise comparisons will be performed using the Bonferroni adjustment, if there are any main or interaction effects within the two independent variables. The pain scale will be also compared with the same ANOVA model.

Discussion

Many approaches to stimulating residual nerve circuits in individuals with cSCI have been tested. In this study, we propose a new approach using RIC to transiently impede upper extremity blood flow, to determine if RIC alone, or in combination with task specific exercise training, enhances hand function. Furthermore, we investigate the possible mechanism by examining changes in electrophysiology within the motor cortex and corticospinal tract, and gene expression of inflammatory mediators. Importantly, we will carefully monitor cardiovascular parameters and document pain/discomfort to understand hemodynamic stability and tolerability when applying RIC in persons with cSCI. By sharing the details of our NIH-funded research protocol, we hope other interested researchers will seek to investigate similar approaches – depending on overlap with the current study and mutual sharing of participant-level data, this could increase the sample size, power, and generalizability of the analysis and results.

Although RIC has been shown to be safe in the healthy able-bodied population as well as in individuals with heart disease and critically ill patients with subarachnoid hemorrhage[33,34,56,57], there are no data describing the safety of RIC in the SCI population. However, widespread sensory impairment, including a limited ability to feel pain/discomfort, may compromise the safety profile of such techniques in participants with cSCI. In addition, damage to the ANS after cSCI contributes to cardiovascular dysregulation, which may alter hemodynamic responses to RIC. Several studies[58–61] have reported stable HR and BP responses before and after RIC in healthy participants, and also in participants with heart disease or vascular stenosis. BP responses remained unchanged during the course of RIC in able-bodied participants and in participants with stable angina pectoris[58,60]. However, sympathetic hypoactivity after cSCI might result in altered or delayed hemodynamic responses, possibly leading to fluctuation of HR and BP during RIC. These possibilities make it essential to record HR, BP and SaO_2 in real-time and document acute pain/discomfort and any adverse effects during RIC in individuals with cSCI.

Importantly, given the pilot nature of this study, several limitations are anticipated. First, applying RIC to facilitate motor task learning is a relatively new area with little preliminary data. Therefore, our primary

hypothesis that RIC will promote increased corticospinal excitability to hand muscles involved in motor tasks is speculative. Additionally, the effects of RIC on acute post-exercise facilitation have mostly been shown in healthy able-bodied participants and neuronal damage to motor and sensory pathways in individuals with cSCI might diminish responses to RIC and isometric hand exercise compared to reports in non-disabled participants. Heterogeneity in injury level and residual nerve circuits among participants with cSCI might result in wide variation in outcomes. Nevertheless, although our hypotheses are speculative, the proposed study is designed to provide clear answers. If brief isometric hand exercise plus RIC does not improve corticospinal transmission or pinch strength in participants with cSCI, our next step might test RIC in combination with more advanced interventions such as non-invasive paired stimulation, a technique that our research team is actively studying in other protocols (ClinicalTrials.gov Identifier: NCT03414424, NCT02469675 and NCT03806023). Finally, if the pilot study shows a significant synergistic effect of RIC on the isometric hand exercise task, our future goal will be coupling RIC with more prolonged rehabilitation training to promote long-term beneficial effects.

Additionally, using standard gene expression profiling techniques, if we do not observe changes in gene expression of the TLR signaling pathway, the next step would be to use RNA-sequencing (RNA-Seq) to obtain a broader and unbiased gene expression profile. Initial RNA-seq runs could be limited to 8 million reads/sample, 100bp single end, which would yield a general overview of relative changes in gene expression of the high to moderately expressed genes.

In conclusion, this study is the first to test RIC in people with cervical spinal cord injury and investigate whether RIC alters nerve excitability in the pathway from the brain to the spinal cord to intrinsic hand muscles involved in fine motor tasks. If synergistic effects of RIC with physical training are demonstrated in this study, then effects of RIC coupled with various other rehabilitation interventions can be tested in future studies. In addition, we expect the analysis of neurophysiology and inflammatory mediators before and after RIC to provide some preliminary information regarding the mechanism by which RIC promotes neuroplasticity and improves functional training effects. Thus, results from this study will give us the information to apply RIC coupled with rehabilitation interventions to enhance long-term functional movements in people with cervical spinal cord injury.

Abbreviations

ABP
Abductor Pollicis Brevis
FDI
First Dorsal Interosseous
CST
Corticospinal Tract
RIC
Remote Ischemic Conditioning
SCI

Spinal Cord Injury
cSCI
Cervical Spinal Cord Injury
BDNF
Brain-Derived Neurotrophic Factor
AIH
Acute Intermittent Hypoxia
VEGF
Vascular Endothelial Growth Factor
TLR
Toll-like receptor
TNF
Tumor Necrosis Factor
MAP
Mitogen-Activated Protein
ANS
autonomic nervous system
HR
Heart Rate
BP
Blood Pressure
SaO_2
Oxygen Saturation
MEP
Motor Evoked Potential
TMS
Transcranial Magnetic Stimulation
RMT
Resting Motor Threshold
AMT
Active Motor Threshold
MVC
Maximal Voluntary Contraction
ISNCSCI
the International Standards for the Neurological Classification of Spinal Cord Injury
SICI
Short Interval Cortical Inhibition
LICI
Long Interval Cortical Inhibition
ICF

Intra-Cortical Facilitation
CS
Conditioning Stimulus
TS
Test Stimulus
ISI
InterStimulus Intervals

Declarations

Ethics approval and consent to participate

The study protocol has been approved by the institutional review board (IRB) and research and development committee at James J. Peter VA Medical Center. The protocol number is HAR-18-47.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YK, NH, JW, and OB designed the protocol. YK and NH are major contributor in writing the manuscript. All authors read and approved the final manuscript.

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References

1. Center, N. S. C. I. S. *Facts and Figures at a Glance*; The University of Alabama at Birmingham: Birmingham, AL, 2016.
2. Snoek, G. J.; IJzerman, M. J.; Hermens, H. J.; Maxwell, D.; Biering-Sorensen, F. Survey of the Needs of Patients with Spinal Cord Injury: Impact and Priority for Improvement in Hand Function in Tetraplegics. *Spinal cord* **2004**, *42*, 526–32.
3. Anderson, K. D. Targeting Recovery: Priorities of the Spinal Cord-Injured Population. *J Neurotrauma* **2004**, *21*, 1371–1383.
4. Kakulas, A. B. A Review of the Neuropathology of Human Spinal Cord Injury with Emphasis on Special Features. *The Journal of Spinal Cord Medicine* **1999**, *22*, 119–124.
5. Topka, H.; Cohen, L. G.; Cole, R. A.; Hallett, M. Reorganization of Corticospinal Pathways Following Spinal Cord Injury. *Neurology* **1991**, *41*, 1276–83.
6. Behrman, A. L.; Bowden, M. G.; Nair, P. M. Neuroplasticity after Spinal Cord Injury and Training: An Emerging Paradigm Shift in Rehabilitation and Walking Recovery. *Phys Ther* **2006**, *86*, 1406–1425.
7. Edgerton, V. R.; Tillakaratne, N. J.; Bigbee, A. J.; Leon, R. D. de; Roy, R. R. Plasticity of the Spinal Neural Circuitry after Injury. *Annu Rev Neurosci* **2004**, *27*, 145–167.
8. Harel, N. Y.; Strittmatter, S. M. Can Regenerating Axons Recapitulate Developmental Guidance during Recovery from Spinal Cord Injury? *Nat Rev Neurosci* **2006**, *7*, 603–616.
9. Ramer, M. S.; Harper, G. P.; Bradbury, E. J. Progress in Spinal Cord Research - a Refined Strategy for the International Spinal Research Trust. *Spinal cord : the official journal of the International Medical Society of Paraplegia* **2000**, *38*, 449–472.
10. Navarrete-Opazo, A.; Alcayaga, J.; Sepúlveda, O.; Rojas, E.; Astudillo, C. Repetitive Intermittent Hypoxia and Locomotor Training Enhances Walking Function in Incomplete Spinal Cord Injury Subjects: A Randomized, Triple-Blind, Placebo-Controlled Clinical Trial. *Journal of Neurotrauma* **2017**, *34*, 1803–1812.
11. Dirnagl, U.; Becker, K.; Meisel, A. Preconditioning and Tolerance against Cerebral Ischaemia: From Experimental Strategies to Clinical Use. *Lancet Neurol.* **2009**, *8*, 398–412.
12. Gidday, J. M. Cerebral Preconditioning and Ischaemic Tolerance. *Nat. Rev. Neurosci.* **2006**, *7*, 437–448.
13. Stetler, R. A.; Leak, R. K.; Gan, Y.; Li, P.; Zhang, F.; Hu, X.; Jing, Z.; Chen, J.; Zigmond, M. J.; Gao, Y. Preconditioning Provides Neuroprotection in Models of CNS Disease: Paradigms and Clinical Significance. *Prog. Neurobiol.* **2014**, *114*, 58–83.
14. Kharbanda, R. K.; Mortensen, U. M.; White, P. A.; Kristiansen, S. B.; Schmidt, M. R.; Hoschtitzky, J. A.; Vogel, M.; Sorensen, K.; Redington, A. N.; MacAllister, R. Transient Limb Ischemia Induces Remote Ischemic Preconditioning in Vivo. *Circulation* **2002**, *106*, 2881–2883.
15. Kharbanda, R. K.; Nielsen, T. T.; Redington, A. N. Translation of Remote Ischaemic Preconditioning into Clinical Practice. *Lancet* **2009**, *374*, 1557–1565.

16. Ren, C.; Yan, Z.; Wei, D.; Gao, X.; Chen, X.; Zhao, H. Limb Remote Ischemic Postconditioning Protects against Focal Ischemia in Rats. *Brain Research* **2009**, *1288*, 88–94.
17. Lim, S. Y.; Hausenloy, D. J. Remote Ischemic Conditioning: From Bench to Bedside. *Front. Physiol.* **2012**, *3 FEB*.
18. Gill, R.; Kuriakose, R.; Gertz, Z. M.; Salloum, F. N.; Xi, L.; Kukreja, R. C. Remote Ischemic Preconditioning for Myocardial Protection: Update on Mechanisms and Clinical Relevance. *Mol. Cell. Biochem.* **2015**, *402*, 41–49.
19. Correia, S. C.; Moreira, P. I. Hypoxia-Inducible Factor 1: A New Hope to Counteract Neurodegeneration? *J. Neurochem.* **2010**, *112*, 1–12.
20. Albrecht, M.; Zitta, K.; Bein, B.; Wennemuth, G.; Broch, O.; Renner, J.; Schuett, T.; Lauer, F.; Maahs, D.; Hummitzsch, L.; Cremer, J.; Zacharowski, K.; Meybohm, P. Remote Ischemic Preconditioning Regulates HIF-1α Levels, Apoptosis and Inflammation in Heart Tissue of Cardiosurgical Patients: A Pilot Experimental Study. *Basic Research in Cardiology* **2013**, *108*.
21. Konstantinov, I. E.; Arab, S.; Kharbanda, R. K.; Li, J.; Cheung, M. M. H.; Cherepanov, V.; Downey, G. P.; Liu, P. P.; Cukerman, E.; Coles, J. G.; Redington, A. N. The Remote Ischemic Preconditioning Stimulus Modifies Inflammatory Gene Expression in Humans. *Physiological Genomics* **2004**, *19*, 143–150.
22. Kim, Y.-H.; Yoon, D.-W.; Kim, J.-H.; Lee, J.-H.; Lim, C.-H. Effect of Remote Ischemic Post-Conditioning on Systemic Inflammatory Response and Survival Rate in Lipopolysaccharide-Induced Systemic Inflammation Model. *Journal of inflammation (London, England)* **2014**, *11*, 16.
23. Raetz, C. R. H.; Whitfield, C. Lipopolysaccharide Endotoxins. *Annual Review of Biochemistry* **2002**, *71*, 635–700.
24. Lapchak, P. a; Araujo, D. M.; Hefti, F. Systemic Interleukin-1 Beta Decreases Brain-Derived Neurotrophic Factor Messenger RNA Expression in the Rat Hippocampal Formation. *Neuroscience* **1993**, *53*, 297–301.
25. Guan, Z.; Fang, J. Peripheral Immune Activation by Lipopolysaccharide Decreases Neurotrophins in the Cortex and Hippocampus in Rats. *Brain, Behavior, and Immunity* **2006**, *20*, 64–71.
26. Schnydrig, S.; Korner, L.; Landweer, S.; Ernst, B.; Walker, G.; Otten, U.; Kunz, D. Peripheral Lipopolysaccharide Administration Transiently Affects Expression of Brain-Derived Neurotrophic Factor, Corticotropin and Proopiomelanocortin in Mouse Brain. *Neuroscience Letters* **2007**, *429*, 69–73.
27. Herman, P.; Stein, A. B.; Gibbs, K.; Korsunsky, I.; Gregersen, P.; Bloom, O. Persons with Chronic Spinal Cord Injury Have Decreased NK Cell and Increased TLR/Inflammatory Gene Expression. *Journal of Neurotrauma* **2018**.
28. Konstantinov, I. E.; Arab, S.; Kharbanda, R. K.; Li, J.; Cheung, M. M. H.; Cherepanov, V.; Downey, G. P.; Liu, P. P.; Cukerman, E.; Coles, J. G.; Redington, A. N. The Remote Ischemic Preconditioning Stimulus Modifies Inflammatory Gene Expression in Humans. *Physiological genomics* **2004**, *19*, 143–50.
29. Cherry-Allen, K. M.; Gidday, J. M.; Lee, J.-M.; Hershey, T.; Lang, C. E. Remote Limb Ischemic Conditioning Enhances Motor Learning in Healthy Humans. *Journal of Neurophysiology* **2015**, *113*,

3708–3719.

30. Floyd, A. G.; Yu, Q. P.; Piboolnurak, P.; Tang, M. X.; Fang, Y.; Smith, W. A.; Yim, J.; Rowland, L. P.; Mitsumoto, H.; Pullman, S. L. Transcranial Magnetic Stimulation in ALS: Utility of Central Motor Conduction Tests. *Neurology* **2009**, *72*, 498–504, PMCID: PMC2677511.
31. Bøtker, H. E.; Kharbanda, R.; Schmidt, M. R.; Bøttcher, M.; Kaltoft, A. K.; Terkelsen, C. J.; Munk, K.; Andersen, N. H.; Hansen, T. M.; Trautner, S.; Lassen, J. F.; Christiansen, E. H.; Krusell, L. R.; Kristensen, S. D.; Thuesen, L.; Nielsen, S. S.; Rehling, M.; Sørensen, H. T.; Redington, A. N.; Nielsen, T. T. Remote Ischaemic Conditioning before Hospital Admission, as a Complement to Angioplasty, and Effect on Myocardial Salvage in Patients with Acute Myocardial Infarction: A Randomised Trial. *The Lancet* **2010**, *375*, 727–734.
32. Hausenloy, D. J.; Mwamure, P. K.; Venugopal, V.; Harris, J.; Barnard, M.; Grundy, E.; Ashley, E.; Vichare, S.; Salvo, C. Di; Kolvekar, S.; Hayward, M.; Keogh, B.; MacAllister, R. J.; Yellon, D. M. Effect of Remote Ischaemic Preconditioning on Myocardial Injury in Patients Undergoing Coronary Artery Bypass Graft Surgery: A Randomised Controlled Trial. *Lancet* **2007**, *370*, 575–579.
33. Koch, S.; Katsnelson, M.; Dong, C.; Perez-Pinzon, M. Remote Ischemic Limb Preconditioning after Subarachnoid Hemorrhage: A Phase Ib Study of Safety and Feasibility. *Stroke* **2011**, *42*, 1387–1391.
34. Meng, R.; Asmaro, K.; Meng, L.; Liu, Y.; Ma, C.; Xi, C.; Li, G.; Ren, C.; Luo, Y.; Ling, F.; Jia, J.; Hua, Y.; Wang, X.; Ding, Y.; Lo, E. H.; Ji, X. Upper Limb Ischemic Preconditioning Prevents Recurrent Stroke in Intracranial Arterial Stenosis. *Neurology* **2012**, *79*, 1853–1861.
35. Koch, S.; Gonzalez, N. Preconditioning the Human Brain: Proving the Principle in Subarachnoid Hemorrhage. *Stroke* **2013**, *44*, 1748–1753.
36. Cherry-Allen, K. M.; Gidday, J. M.; Lee, J. M.; Hershey, T.; Lang, C. E. Remote Limb Ischemic Conditioning at Two Cuff Inflation Pressures Yields Learning Enhancements in Healthy Adults. *Journal of Motor Behavior* **2017**, *49*, 337–348.
37. Geevasinga, N.; Menon, P.; Kiernan, M. C.; Vucic, S. Motor Cortical Function and the Precision Grip. *Physiological Reports* **2014**, *2*.
38. Rittig-Rasmussen, B.; Kasch, H.; Fuglsang-Frederiksen, A.; Jensen, T. S.; Svensson, P. Specific Neck Training Induces Sustained Corticomotor Hyperexcitability as Assessed by Motor Evoked Potentials. *Spine* **2013**, *38*, E979–E984.
39. Balbi, P.; Perretti, A.; Sannino, M.; Marcantonio, L.; Santoro, L. Postexercise Facilitation of Motor Evoked Potentials Following Transcranial Magnetic Stimulation: A Study in Normal Subjects. *Muscle & Nerve* **2002**, *25*, 448–452.
40. Samii, A.; Wassermann, E. M.; Ikoma, K.; Mercuri, B.; Hallett, M. Characterization of Postexercise Facilitation and Depression of Motor Evoked Potentials to Transcranial Magnetic Stimulation. *Neurology* **1996**, *46*, 1376–1382.
41. Nuzzo Effects of Acute Isometric Resistance Exercise on Cervicomedullary Motor Evoked Potentials. *ARPN Journal of Engineering and Applied Sciences* **2017**, *12*, 3218–3221.

42. Latella, C.; Teo, W. P.; Harris, D.; Major, B.; VanderWesthuizen, D.; Hendy, A. M. Effects of Acute Resistance Training Modality on Corticospinal Excitability, Intra-Cortical and Neuromuscular Responses. *European Journal of Applied Physiology* **2017**, *117*, 2211–2224.
43. Nørgaard, P.; Nielsen, J. F.; Andersen, H. Post-Exercise Facilitation of Compound Muscle Action Potentials Evoked by Transcranial Magnetic Stimulation in Healthy Subjects. *Experimental Brain Research* **2000**, *132*, 517–22.
44. Jensen, J. L.; Marstrand, P. C. D.; Nielsen, J. B. Motor Skill Training and Strength Training Are Associated with Different Plastic Changes in the Central Nervous System. *Journal of applied physiology (Bethesda, Md.: 1985)* **2005**, *99*, 1558–1568.
45. Chen, R.; Cros, D.; Curra, A.; Lazzaro, V. Di; Lefaucheur, J. P.; Magistris, M. R.; Mills, K.; Rosler, K. M.; Triggs, W. J.; Ugawa, Y.; Ziemann, U. The Clinical Diagnostic Utility of Transcranial Magnetic Stimulation: Report of an IFCN Committee. *Clin Neurophysiol* **2008**, *119*, 504–532.
46. McGie, S. C.; Masani, K.; Popovic, M. R. Failure of Spinal Paired Associative Stimulation to Induce Neuroplasticity in the Human Corticospinal Tract. *37*, 565–574.
47. Thabit, M. N.; Ueki, Y.; Koganemaru, S.; Fawi, G.; Fukuyama, H.; Mima, T. Movement-Related Cortical Stimulation Can Induce Human Motor Plasticity. *30*, 11529–11536.
48. Vaseghi, B.; Zoghi, M.; Jaberzadeh, S. Inter-Pulse Interval Affects the Size of Single-Pulse TMS-Induced Motor Evoked Potentials: A Reliability Study. *Basic and Clinical Neuroscience* **2015**.
49. Chen, R.; Tam, A.; Bütefisch, C.; Corwell, B.; Ziemann, U.; Rothwell, J. C.; Cohen, L. G. Intracortical Inhibition and Facilitation in Different Representations of the Human Motor Cortex. *Journal of Neurophysiology* **1998**.
50. Carson, R. G.; Nelson, B. D.; Buick, A. R.; Carroll, T. J.; Kennedy, N. C.; Cann, R. Mac Characterizing Changes in the Excitability of Corticospinal Projections to Proximal Muscles of the Upper Limb. *Brain Stimulation* **2013**.
51. Chen, R.; Cros, D.; Curra, A.; Lazzaro, V. Di; Lefaucheur, J.-P.; Magistris, M. R.; Mills, K.; Rösler, K. M.; Triggs, W. J.; Ugawa, Y.; others The Clinical Diagnostic Utility of Transcranial Magnetic Stimulation: Report of an {IFCN} Committee. *119*, 504–532.
52. Kujirai, T.; Caramia, M. D.; Rothwell, J. C.; Day, B. L.; Thompson, P. D.; Ferbert, A.; Wroe, S.; Asselman, P.; Marsden, C. D. Corticocortical Inhibition in Human Motor Cortex. *The Journal of Physiology* **1993**, *471*, 501–519.
53. Du, X.; Summerfelt, A.; Chiappelli, J.; Holcomb, H. H.; Hong, L. E. Individualized Brain Inhibition and Excitation Profile in Response to Paired-Pulse TMS. *Journal of Motor Behavior* **2014**, *46*, 39–48.
54. McNeil, C. J.; Martin, P. G.; Gandevia, S. C.; Taylor, J. L. Long-Interval Intracortical Inhibition in a Human Hand Muscle. *Experimental Brain Research* **2011**, *209*, 287–297.
55. Kobayashi, M.; Pascual-Leone, A. Transcranial Magnetic Stimulation in Neurology. *The Lancet Neurology* **2003**, *2*, 145–156.
56. Connolly, M.; Bilgin-Freiert, A.; Ellingson, B.; Dusick, J. R.; Liebeskind, D.; Saver, J.; Gonzalez, N. R. Peripheral Vascular Disease as Remote Ischemic Preconditioning, for Acute Stroke. *Clinical*

Neurology and Neurosurgery **2013**, *115*, 2124–2129.

57. England, T. J.; Hedstrom, A.; O'Sullivan, S.; Donnelly, R.; Barrett, D. A.; Sarmad, S.; Sprigg, N.; Bath, P. M. RECAST (Remote Ischemic Conditioning after Stroke Trial): A Pilot Randomized Placebo Controlled Phase II Trial in Acute Ischemic Stroke. *Stroke* **2017**, *48*, 1412–1415.
58. Lambert, E. A.; Thomas, C.; Hemmes, R.; Eikelis, N.; Pathak, A.; Schlaich, M. P.; Lambert, G. W. Sympathetic Nervous Response to Ischemia-Reperfusion Injury in Man Is Altered with Remote Ischemic Preconditioning. *American journal of physiology. Heart and circulatory physiology* **2016**, ajpheart.00369.2016.
59. Enko, K.; Nakamura, K.; Yunoki, K.; Miyoshi, T.; Akagi, S.; Yoshida, M.; Toh, N.; Sangawa, M.; Nishii, N.; Nagase, S.; Kohno, K.; Morita, H.; Kusano, K. F.; Ito, H. Intermittent Arm Ischemia Induces Vasodilatation of the Contralateral Upper Limb. *Journal of Physiological Sciences* **2011**, *61*, 507–513.
60. Li, S.; Ma, C.; Shao, G.; Esmail, F.; Hua, Y.; Jia, L.; Qin, J.; Ren, C.; Luo, Y.; Ding, Y.; Borlongan, C. V.; Ji, X. Safety and Feasibility of Remote Limb Ischemic Preconditioning in Patients with Unilateral Middle Cerebral Artery Stenosis and Healthy Volunteers. *Cell Transplantation* **2015**, *24*, 1901–1911.
61. Zagidullin, N.; Scherbakova, E.; Safina, Y.; Zulkarneev, R.; Zagidullin, S. The Impact of Remote Ischemic Preconditioning on Arterial Stiffness and Heart Rate Variability in Patients with Angina Pectoris. *Journal of Clinical Medicine* **2016**, *5*, 60.

Figures

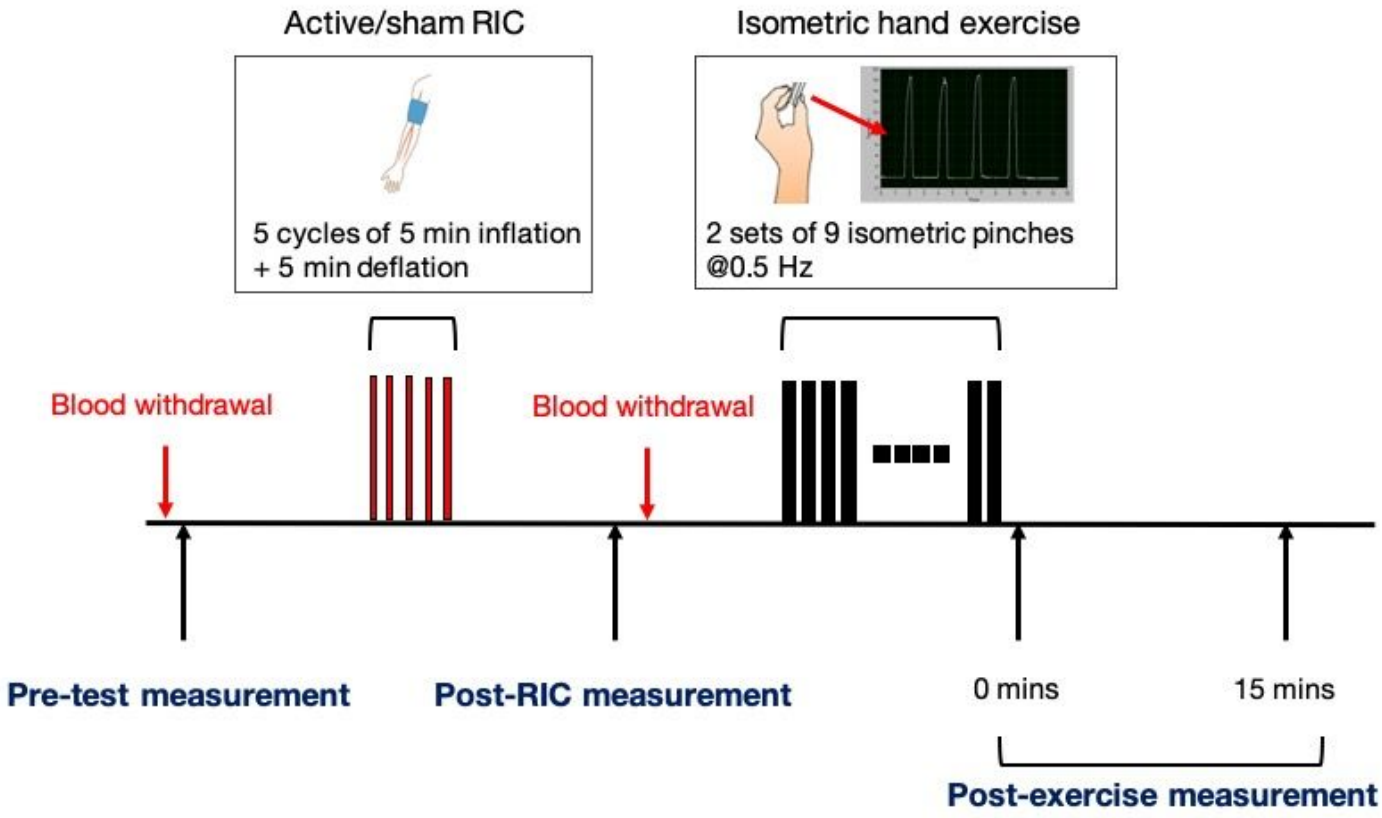

Figure 1

The experimental protocol.

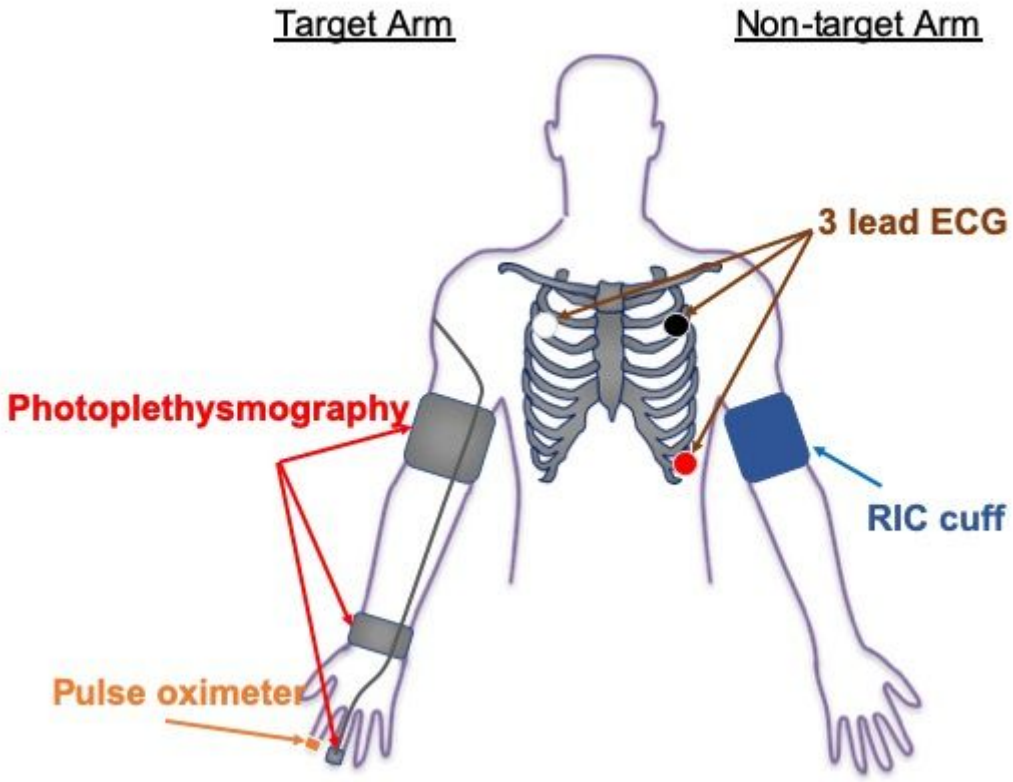

Figure 2

The equipment configuration for testing HR variability, respiratory rate and blood pressure changes during RIC/Sham conditioning.

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